

## The Determination of Zn(II) Ligation in the Active Site of Mammalian Porphobilinogen Synthase by X-ray Absorption Spectroscopy

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Porphobilinogen synthase (PBGS), also known as 5-aminolevulinate (5-ALA) dehydratase, is a homooctameric enzyme of the biosynthetic pathway to porphyrins and corrins. Mammalian PBGS has four active sites per octamer and binds up to 8 Zn/octamer, although only 4 Zn/octamer are needed for maximal activity. We have examined the Zn K-edge X-ray absorption spectrum (XAS) of several samples of human PBGS isolated from an *E. coli* expression system using a synthetic gene. Only very minor changes in the spectra are induced by different zinc loadings (4 or 8 Zn/octamer) and presence or absence of product (PBG formed *in situ* by reaction of ALA just prior to freezing of sample). The similarity of the spectra in the 4 and 8 Zn/octamer samples contrasts with the significantly different spectra observed in a previous XAS study of bovine liver PBGS (Dent, A.J.; Beyersmann, D.; Block, C.; Hasnain, S.S. *Biochemistry* 29, 7822-7828, 1990). Our analysis of the EXAFS of the human PBGS samples (regardless of zinc loading) is consistent with an average coordination of zinc atoms by 3 sulfur atoms at 2.3 Å and one nitrogen and/or oxygen atom at 2.0 Å. We recently began EXAFS studies of various mutant and/or chemically modified forms of the human enzyme.